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3D Blood–Brain Barrier Rat Model

Product Information:

Our 3D Blood–Brain Barrier Rat Model (BBB) characteristics are induced and maintained by cross-talk between brain microvessel endothelial cells and neighbouring elements of the neurovascular unit. We have developed and characterized a new syngeneic BBB model using primary cultures of the three main cell types of cerebral microvessels. The co-culture of rat endothelial cells, pericytes and astrocytes mimick the anatomical situation *in vivo*. This model can be used for Drug BBB Permeability study, form micromolecules to cells.

In the presence of both pericytes and astrocytes rat brain endothelial cells expressed enhanced levels of tight junction (TJ) proteins occludin, claudin-5 and ZO-1 with a typical localization at the cell borders. Further morphological evidence of the presence of interendothelial TJs was provided by electron microscopy. The transendothelial electrical resistance (TEER) of brain endothelial monolayers in triple co-culture, indicating the tightness of TJs reached 400 Ω cm^2 on average, while the endothelial permeability coefficients (P_e) for fluorescein was in the range of 3×10^{-6} cm/s. Brain endothelial cells in the new model expressed glucose transporter-1, efflux transporters P-glycoprotein and multidrug resistance protein-1, and showed a polarized transport of rhodamine 123, a ligand for P-glycoprotein. To further characterize the model, drug permeability assays were performed using a set of compounds with known *in vivo*BBB permeability. The new BBB model, which is the first model to incorporate pericytes in a triple co-culture setting, can be a useful tool for research on BBB physiology and pathology and to test candidate compounds for centrally acting drugs. This model is ready to use, and easily transportable.

Our *in vitro 3D rat* model of the blood-brain barrier (BBB) made of primary cultures of rat (Wistar rat) brain capillary endothelial cells, brain pericytes and astrocytes. With a polyethylene terephthalate (PT) membrane is a ready-to-use concept for your evaluations of BBB-permeability of drugs and drug candidates.

The model comes in the following formats:

- 6-well format construct
- 12-well format construct
- 24-well format construct
- 96-well format construct

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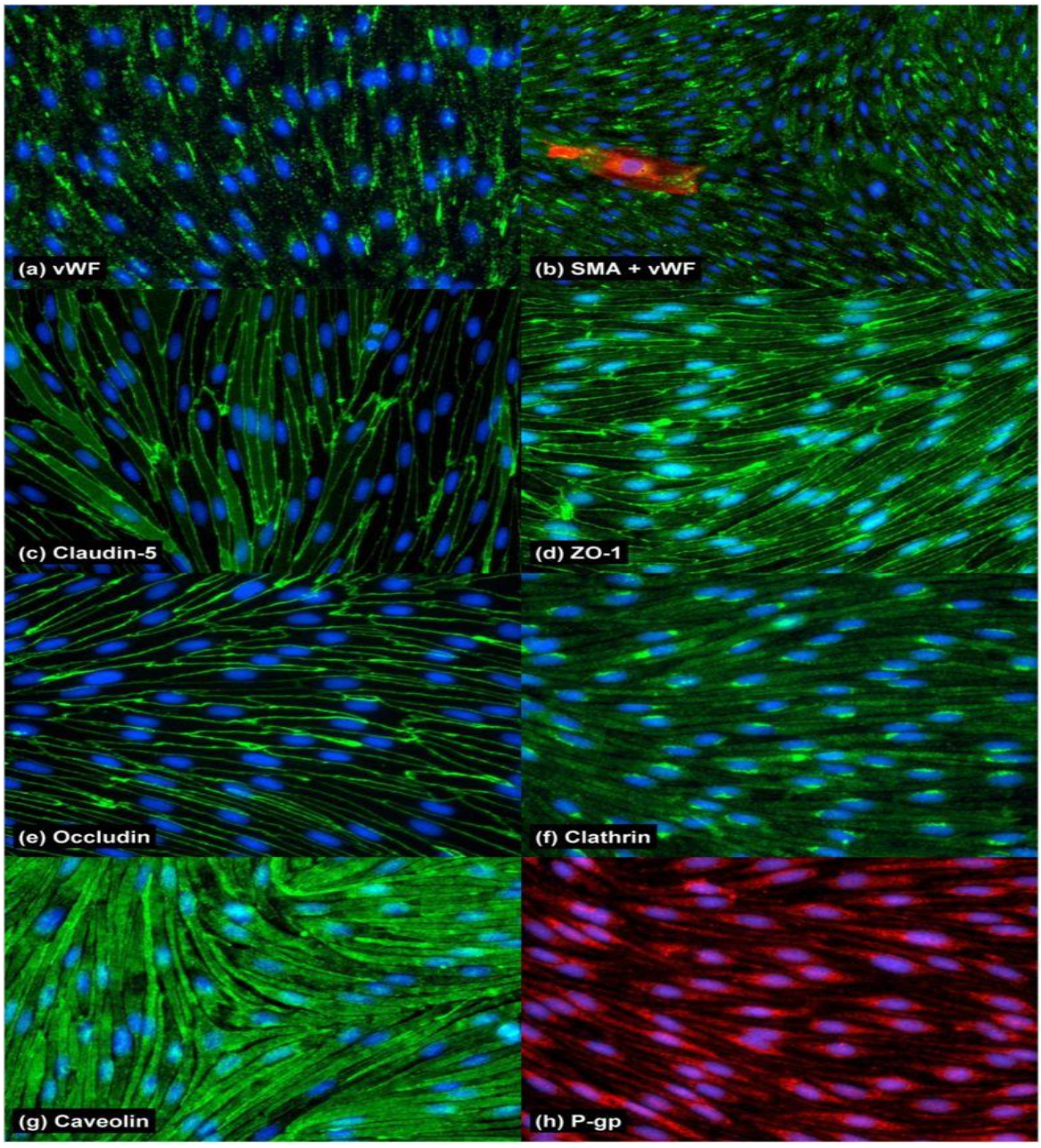
For evaluating the permeability of a molecule through the brain endothelial and pericytes layers of our model, in blood-to-brain direction, the molecule is applied to the upper compartment of the insert. Cells within are not clearly seen by microscopic observations.

We deliver the complete set of 3D BBB Rat model in frozen packaged with dry ice. It can be frozen as a whole and stored at -80℃. We guarantee the use of our model within 1 month.

Characterization of 3D BBB Rat Model by immunocytochemistry



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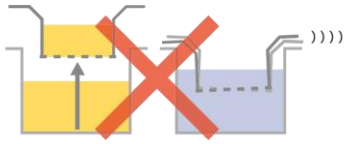
(Move frozen thawing-solution **(media 1)** to 37 ℃ water-bath.)

2. Move thawing-sol to clean-bench.

3. Move a BBB Kit in frozen to clean-bench. Take off seals. (Do not take a minute.)

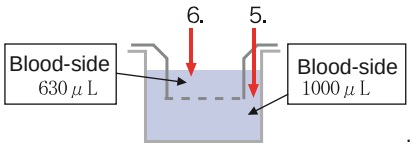
4. Wipe up waterdrops (humidity) on BBB Model with clean papers.

5. Add 1,000 μl thawing-sol to Brain-side (to all 12-wells), through an opening between Inserts.



Do not touch membrane of insert with pipette, and do not move insert, during procedures of #1 to #5.

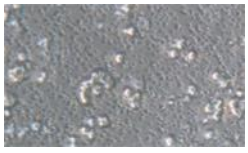
6. Add 630 μl thawing-sol to Blood-side (inside of Insert) (to all 12-wells).

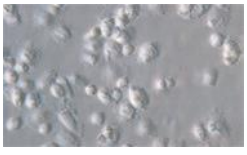


7. Stir up gently Blood-side (inside of Insert) with a pipette, 5 to 10 times.

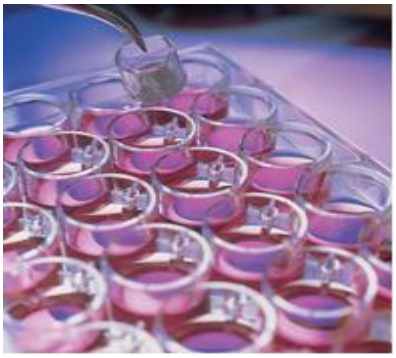
8. Incubate BBB Kit for 2 to 3 hrs in CO_2 incubator. During this incubation, warm medium 1 to 37 ℃

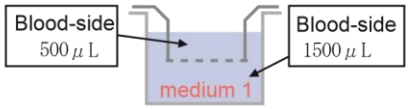
9. Check cells with inverted microscope.





cells, carelessly. Add medium 1, very gently.)

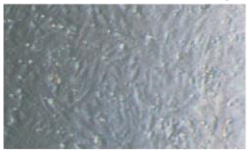




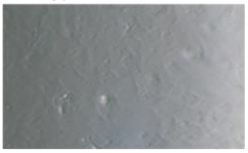
11. Incubate BBB Kit with medium 1 in CO_2 incubator, overnight.

[On day 1 (the next day after thawing of BBB kit)]

12 Check cells to be monolayer (confluency) with inverted microscope.



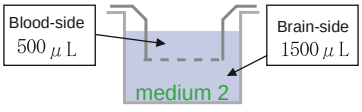
endothelial cells (low magnification)



astrocytes (low magni)

13. (Move frozen medium 2 to 37 ℃ water-bath.)

14. Wipe up humidity on surface and bottom of BBB Kit.Remove medium 1 from Brain-side, and add 1,500 μL medium 2 (Do not stir up Brain-side.)



(red arrow), then remove medium 1 from Blood-side, and

15. add 500 μL medium 2. (Do not touch cells, carelessly. Add medium 2, very gently.)

16. Incubate BBB with medium 2 in CO_2 incubator for 3 days. (from thawing day (Day) to Day 4)On Day 4 BBB is activated functionally, and maintains BBB function until day 7. Use activated-BBB t on Day 4.(You can store activated-BBB in CO_2 incubator at 37 ℃. We recommend you use the BBB until Day 6.)

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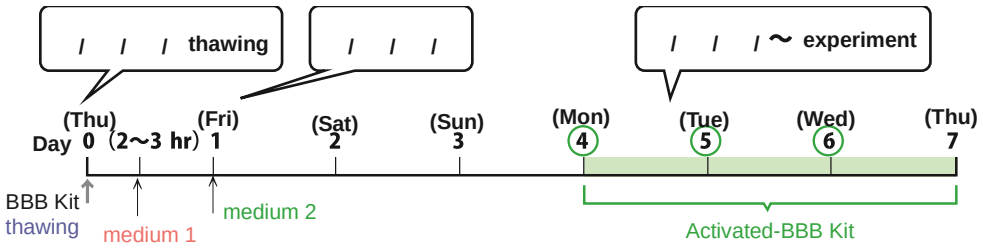
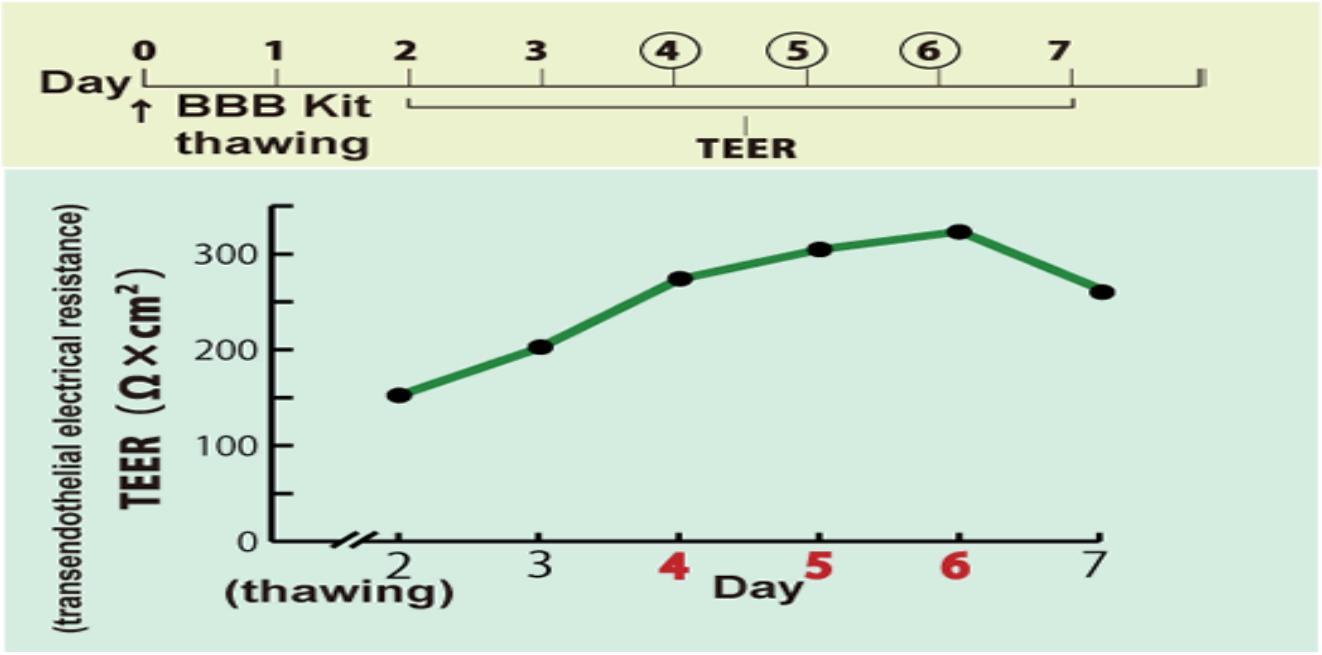
Schedule
(example)



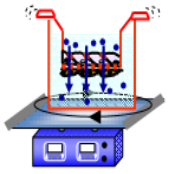


Transendothelial electrical resistance (TEER)

TEER was measured by EVOM resistance meter (World Precision Instruments). TEER depends on the voltage between electrodes across RBEC monolayer, which reflects an amount of ionic molecule flux through RBEC monolayer.

Transcellular transport and paracellular transport

Permeability of drugs across RBEC monolayer was determined as previously described (Kis et al., 2001).



$$\frac{1}{PS_e} = \frac{1}{PS_{total}} - \frac{1}{PS_{mem}}$$

$$P_e\,(cm/min) = \frac{PS_e}{A}$$

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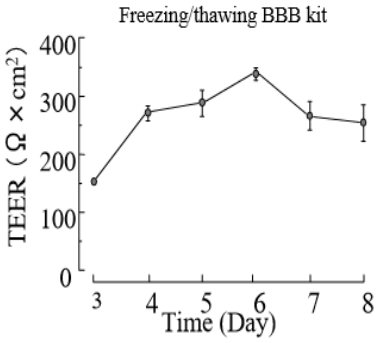
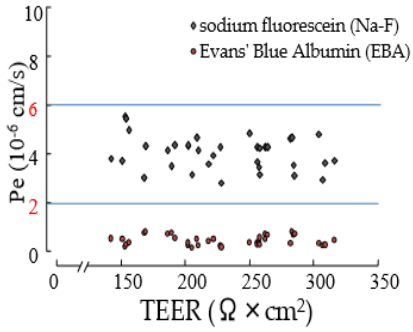
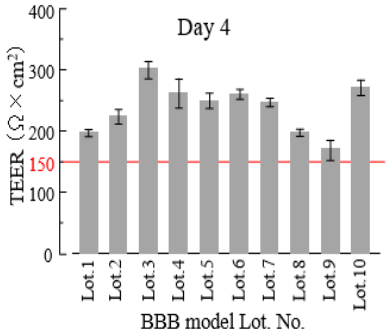
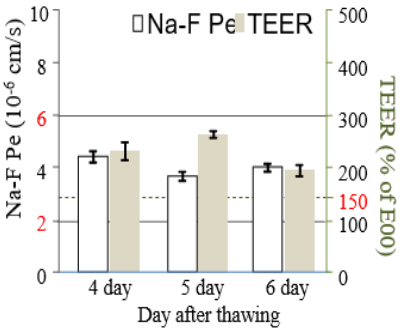








■ TEER of RBEC monolayer, indicating the tightness of interendothelial tight junctions (TJs), was gradually increased to the level of 250 Ω × cm² at day 4, and reached a plateau until 8 day.

■ We found no changes in endothelial permeability coefficients (Pe) for sodium fluorescein (Na-F) among kits with TEERs of 150 to 320 Ω × cm². There were no differences in Pe of RBEC monolayer in triple co-culture tested on days 4, 5 and 6.

name	MW	CNS	transport	Recovery rate (%)
risperidone	410	+	efflux	69.8
fluvoxamine	434	+	lipophil and high protein binding	63.6
trazodone	408	+	passive lipophilic	63.1
fluoxetine	346	+	lipophil and high protein binding	62.3
hydroxyzine	448	+	passive lipophilic	53
haloperidol	376	+	passive lipophilic	52.1
vincristine	923	-	efflux	64.6
digoxin	781	-	efflux	62.7
prazosin	420	-	efflux: ABCG2	57.7
vinblastine	909	-	efflux	53.5
verapamil	491	-	efflux	51.2
nortriptyrine	300	+	Influx: NET	44.4
paroxetine	375	+	lipophil and high protein binding, Pgp inhibitor but not Pgp substrate, lipid soluble	39.7
buspirone	422	+	passive hydrophilic	39.4
chlorpromazine	355	+	efflux, Pgp substrate/inhibitor	32.3
sertraline	343	+	lipophil and high protein binding, Pgp inhibitor	18.7
paclitaxel	854	-	efflux	38.4
loperamide	514	-	efflux	38.3
loratadine	383	-	efflux	16
amiodarone	682	-	efflux	4
cyclosporin	1,203	-	efflux	1.6

Drug permeability assays were done using the BBB kit.
A set of 40 compounds and drugs with known BBB permeability has been tested.

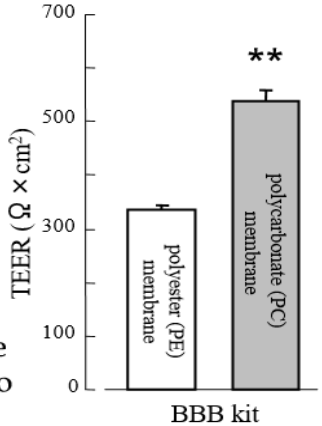
$$\text{Recovery rate (\%)} = \frac{[\text{lu minal}^{t=60}] + [\text{abluminal}^{t=60}]}{[\text{luminal}^{t=0}]} \times 100$$

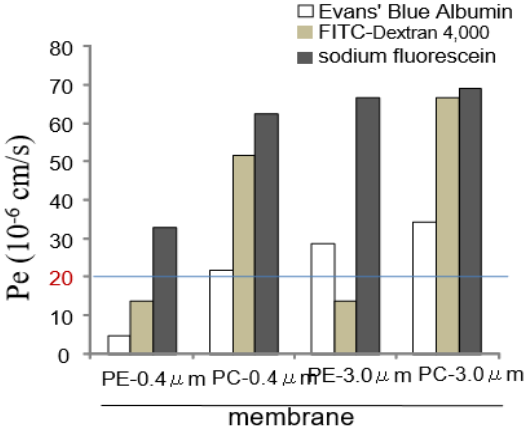
The recovery rates of some compounds were very low, probably because of there adsorption to the insert membranes, Transwell®, and/or pipet tips, or water solubility of the compounds.

Transwell® Permeable Supports

#	membrane	Pore size	Pore density
3460	Polyester (PE)	0.4mm	4× 10⁸ pores/cm²
3401	Polycarbonate (PC)	0.4mm	1× 10⁸ pores/cm²
3462	Polyester (PE)	3.0mm	2× 10⁸ pores/cm²
3402	Polycarbonate (PC)	3.0mm	2× 10⁸ pores/cm²

■ The BBB kit with 3.0 μm pore polycarbonate insert membrane, a candidate kit for macromolecular compounds, had also a significant high level of TEER.





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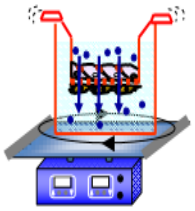
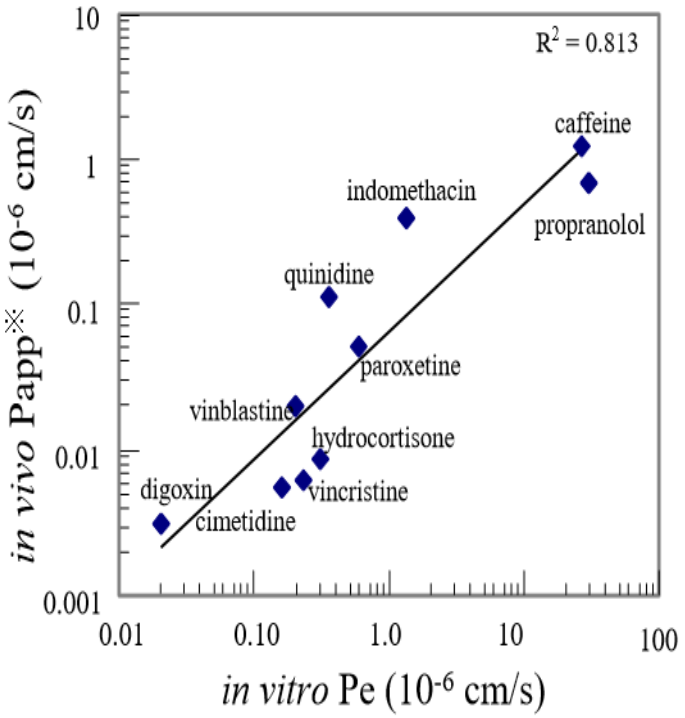
name	MW	CNS	transport	Recovery rate (%)
sulpiride	341	±	efflux: Pgp, influx: OCTN1, OCTN2, PEPT1	91.7
phenytoin	252	+	lipophil and high protein binding	99.6
antipyrin	188	+	passive lipophilic	90
carisoprodol	260	+	passive lipophilic	89.6
indomethacin	358	+	passive hydrophilic	87.6
caffeine	212	+	passive lipophilic	87.5
carbamazepine	236	+	passive lipophilic	85.1
midazolam	326	+	passive lipophilic, highly permeable, Pgp substrate	84.2
propranolole	296	+	passive lipophilic	81.9
zolpidem	382	+	passive lipophilic	71.7
atenolol	226	-	passive hydrophilic, weak base	97.1
cimetidine	252	-	efflux	95.4
acetylsalicilic acid	180	-	organic anion, efflux (Oat1, Oat2)	94.2
epinastine	286	-	efflux	94
prednisone	358	-	efflux (Pgp)	93.2
fexofenadine	538	-	efflux: Pgp, OATP1A2	93.1
hydrocortisone	362	-	efflux (Pgp)	88.2
warfarin	346	-	lipophil and high protein binding	79.8
quinidine	783	-	efflux	72.8

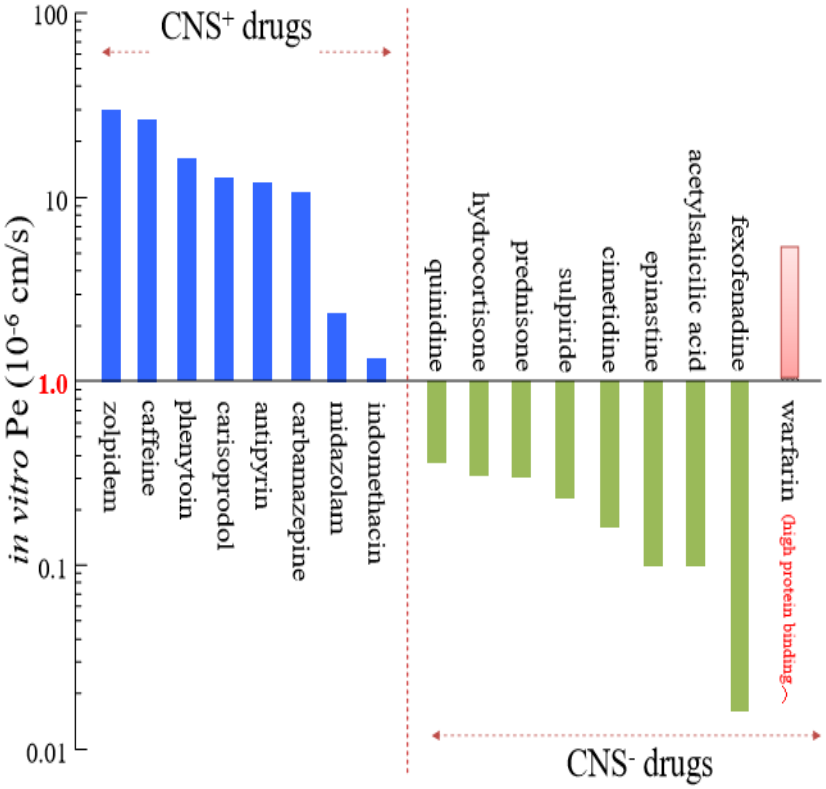
We examined the reliability of *in vitro* permeability data of drugs obtained with the BBB kit.



$$\frac{1}{PS_e} = \frac{1}{PS_{total}} - \frac{1}{PS_{mem}}$$

$$P_e\,(cm/min) = \frac{PS_e}{A}$$





※The value of *in vivo* Papp was obtained from the literature.

- We obtained very good correlation between the BBB kit and *in vivo* permeabilities of drugs.